TEST OF THE EFFECT OF DISTURBANCE ON CIGUATERA IN TUVALU

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We describe a field study on the potential link between the occurrence and intensity of ciguatera outbreaks and human disturbance of coral reefs. We focused on the atolls Niutao, Nui and Nanumea in the Tuvalu group; each having a different history of ciguatera. Relatively small-scale disturbances associated with ship wrecks; and moderate disturbance associated with blasting for boat channels were examined. Increases in *Gambierdiscus toxicus* abundances were detected around channels at Nanumea and Niutao (prior history of outbreak), but not at Nui (historically ciguatera free). At Niutao, the overriding pattern of *G. toxicus* density around the island was independent of either form of human disturbance. Fish toxicity (Hokama Stick Test) data indicated a similar pattern unrelated to human disturbance. We suggest that some forms of human disturbance might affect (or even precipitate) outbreaks of ciguatera but that other factors are likely to play a larger role.

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Ciguatera poisoning from coral reef fishes is a problem for Pacific Island countries, both for expansion of subsistence fishing to support increasing populations, and for inshore commercial fisheries (Dalzell,1992; Lewis, in press). While knowledge has increased on the toxins involved (Murata et al.,1990; Lewis et al.,1991), the dinoflagellates responsible for ciguatoxins (*Gambierdiscus toxicus* and others) (Lewis et al. 1991; Holmes & Lewis,1991; Holmes et al.,1991), and the fish responsible for intoxications (Anderson & Lobel,1987; Dalzell,1992), the causes of outbreaks elude us. The spatial and temporal patterns in the abundance of *G. toxicus* and ciguatera poisonings paint a complex picture. Some areas appear never to have outbreaks. Other areas show severe short-term but unpredictable outbreaks lasting a few years (Kaly et al.,1991), hereafter 'acute outbreaks'. Other 'hot spots' have had ciguatera problems for decades, sometimes with a marked seasonality (Carlson & Tindall,1985; Gillespie et al.,1985a), hereafter 'chronic outbreaks'. Field data of dinoflagellate numbers relative to ciguatera outbreaks are rare.

Speculations on the cause(s) of ciguatera outbreaks include natural and human-induced phenomena. Anecdotal and correlative studies have implicated high salinity (Yasumoto et al., 1980b; Carlson,1984; Taylor,1985), low rainfall (Carlson & Tindall,1985), low storm or wave activity (Taylor & Gustavson 1985), high levels of nutrients (Carlson,1984), physical destruction of reefs, both natural and man-made (Randall, 1958; Banner,1976) coral death or bleaching (Yasumoto et al.,1980a,b; Kohler & Kohler, 1992) and even nuclear testing (Bagnis,1969). However, no one factor provides a universal explanation and there have been few attempts to test specific hypotheses.

The notion that the physical destruction of reef areas promotes ciguatera, is based on the idea that freshly denuded surfaces are colonised by certain opportunistic macroalgae that are the preferred hosts for the epiphytic dinoflagellates responsible for ciguatera (Randall,1958; Banner,1976). Support comes from studies on a variety of disturbances including the construction of boat channels (Kuberski,1979; Banner,1974; Tebano,1984, 1992), the locations of wrecks or anchorages (Cooper,1964), and storm-induced and other disturbances to the reef system (Bagnis,1969; Bagnis et al.,1985). However, testing the importance of these factors requires the simultaneous sampling of disturbed and undisturbed sites, and preferably, sampling both before and after the disturbance has occurred.

Our study examines potential effects of boat channels and shipwrecks on incidence of ciguatera in Tuvalu. We used 2 field indicators to test the potential effects of these disturbances. Firstly, abundance of *Gambierdiscus toxicus* was measured on a range of host algae. Secondly, we used an index of toxicity of muscle tissue from a surgeonfish (*Ctenochaetus strigosus*) implicated in ciguatera, using the Hokama 'stick' test (Hokama et al.,1983,1987). We assumed that these techniques may independently provide a measure of the levels of ciguatera at different sites or times without there necessarily being a direct

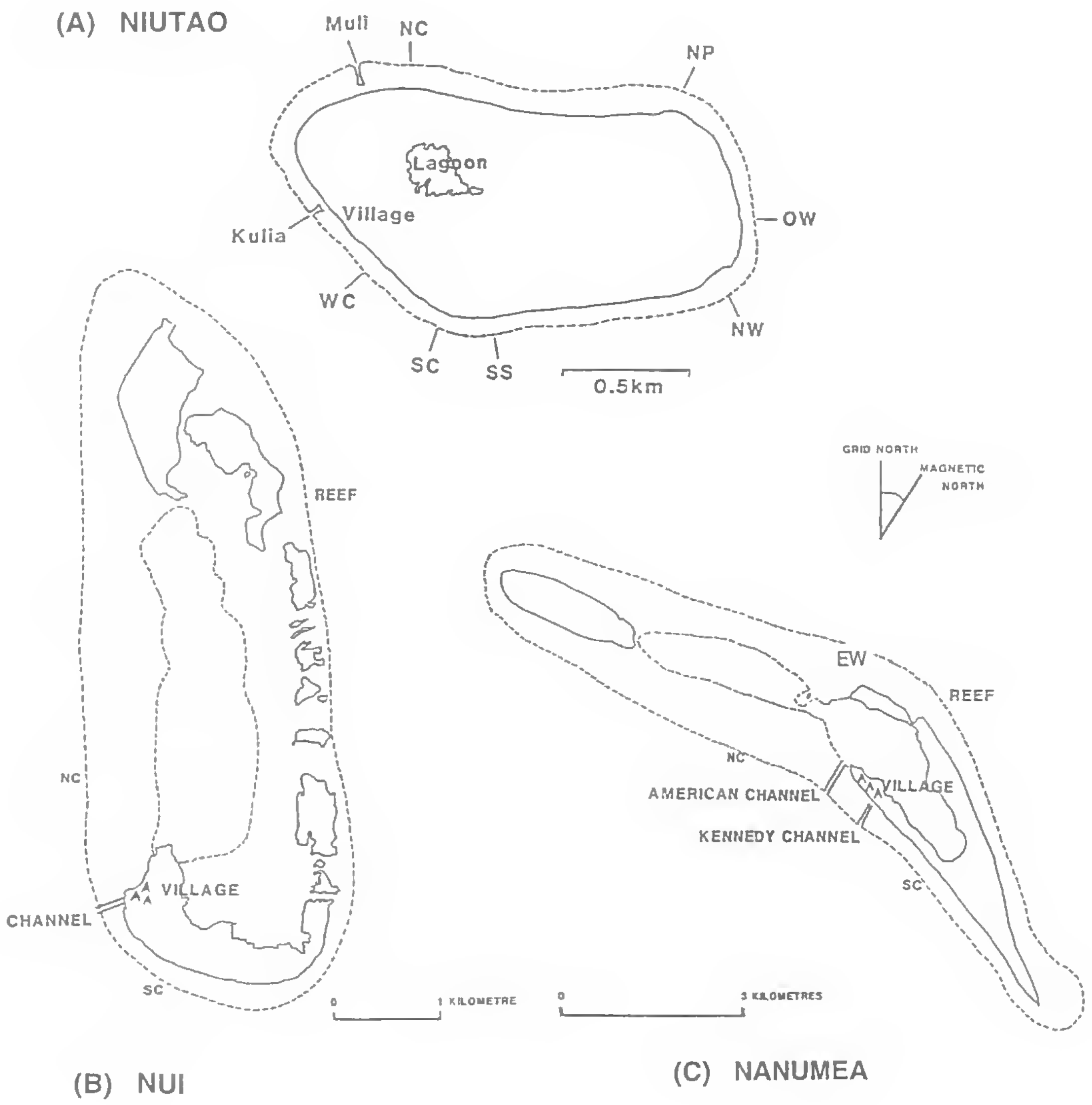

FIG. 1. Maps of (A) Niutao, (B) Nui and (C) Nanumea showing channels, controls and wrecks surveyed. Muli and Kulia are channels, NC=North Control, WC=West Control, SC=South Control, SS=Sagasaga, NP=North Point, NW=New Wreck, OW=Old Wreck, EW=East Wreck.

link between dinoflagellate numbers and fish toxicity.

Sampling protocols to test the effect of disturbance were: (1) a comparison between areas adjacent to pre-existing channels and shipwrecks with control areas, for numbers of *G. toxicus* and for fish toxicity; and (2) a short-term study of changes occurring in response to the construction of two new boat channels, and distant control areas. By sampling different distances from these boat channels and control areas over an 18 month period, we attempted to demonstrate any successional effects on ciguatera that could be attributed unequivocally to boat channels. Since an outbreak of ciguatera began just prior to the construction of the channels on Niutao (Kaly et al., 1991), the study provided a useful test of the

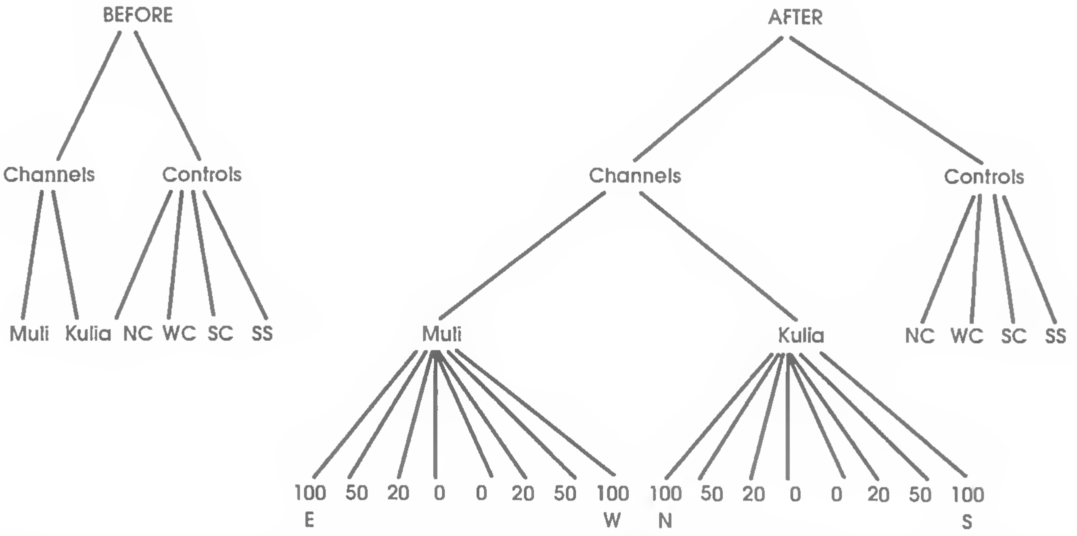

FIG.2. Design of the sampling programme used to detect any impacts of channels on indicators of ciguatera at Niutao. Numbers under each channel *after* construction, are distances away from the channel in metres.

effects of disturbance during a period when fish toxicity was high.

METHODS

STUDY AREAS AND SAMPLING REGIMES

Construction of boat channels and ship wrecks represent two of the most obvious forms of human disturbance to coral reef platforms and the shallow subtidal zone in Tuvalu. This study focussed on pre-existing boat channels on Nanumea (2 channels) and Nui (1 channel), pre-existing ship wrecks on Nanumea (1 wreck) and Niutao (2 wrecks), and two new boat channels constructed on Niutao during the study. These islands differed in history of recorded ciguatera outbreaks, with none 'ever recorded' from Nui, an area on Nanumea which appears to have been toxic for many years, and a severe outbreak which occurred on Niutao and recovered over a 2-4 year period.

The Nui channel and two control sites on that island were sampled during early September 1989 (Fig.1B). The two channels (American and Kennedy), two control sites and the wreck site on Nanumea were sampled in late September 1989 (Fig. 1C). On Niutao, 2 wreck sites (NW and OW) and 4 control sites (NC, Muli, WC and NP) were sampled prior to construction of any boat channels during May 1989 (Fig.1A).

The 2 future channel sites (Kulia and Muli) and 4 controls (NC, WC, SS, SC) (Fig.1A) were sampled prior to construction of the channels during January 1989 (Fig.2 for sampling design). Sampling at these sites was repeated during channel construction (May 1989), 3 months after channel construction (September 1989) and 15 months after channel construction (September 1990). Sampling was carried out adjacent to each channel, 20m, 50m and 100m in both directions away from the channels, to assess spatial extent of any impacts on the abundance of *G. toxicus.*

MEASUREMENT OF *GAMBIERDISCUS* ABUNDANCE AND COVER OF HOST ALGAE

The distribution and abundance of *Gambierdiscus toxicus*, the only dinoflagellate potentially responsible for the toxin that was found in appreciable numbers in our study, was sampled using a method similar to Yasumoto et al. (1980a). This is a simple washing procedure to separate dinoflagellate from host macroalga. It has been used successfully in field studies, with minor modifications, by other workers (Tebano & McCarthy,1984; Tebano,1984). Weighed samples of 100g of each algal species were placed in a plastic container to which filtered seawater was added. The contents of the container were then shaken vigorously for 2 min (c.250 shakes) before being sieved at 1mm and 38μm. The residue on the 38μm sieve was then washed into a 50ml vial to which 5ml of concentrated formalin

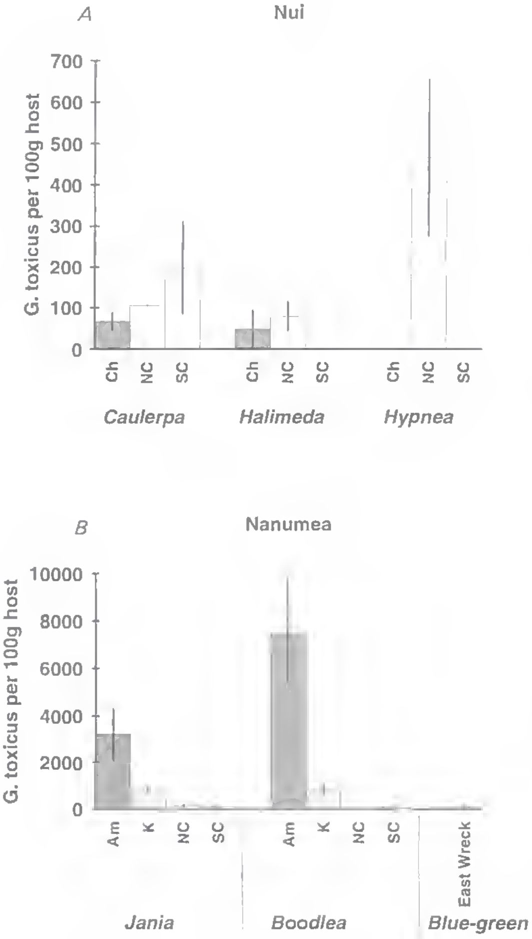

FIG.3. Mean abundance of *G. toxicus* at Channels and Controls at (A) Nui and (B) Nanumea (Time 3 = October 1989). Values are mean cells counts ± standard error. Ch=Nui Channel; NC=North Control; SC=South Control; Am=American Channel; K=Kennedy Channel.

had been added. Samples were brought back to the laboratory, quickly shaken to resuspend particles, and allowed to settle for several days. After settlement, each vial was found to contain a basal sediment layer, a layer of partially suspended mainly organic matter and an uppermost layer of transparent formalin and seawater solution. Neither the formalin solution nor the sedimentary layer were found to contain *G. toxicus* that would have been alive at the time of collection (there were some skeletons in the sedimentary layer).

To estimate the abundance of *G. toxicus* in a sample, the thickness of the primarily organic layer was measured in the pre-settled but undisturbed sample jar using vernier callipers (correct to 0.1mm) for later determination of volume. Five replicate 0.1ml subsamples of the organic layer were drawn from each jar using a micro-pipette and mounted individually on a microscope slide. All cells of *G. toxicus* on each slide were counted with the 5 replicates and volume measurement being used to obtain an estimate of the total number of cells in the organic layer of the sample (and hence the whole jar). This value gave abundances of cells per 100g of host alga.

Three replicate samples of 100g of 2–3 species of intertidal host algae were collected from each sampling site and time. The identity of the host species varied among islands and sites according to what was available. On Nui, *Caulerpa*, *Halimeda* and *Hypnea* were sampled; on Nanumea, *Jania*, *Boodlea* and an unidentified blue-green alga were sampled. On Niutao, *Jania*, *Caulerpa* and an unidentified green turfing alga were collected. To convert estimates of cell numbers per 100g of individual host algae to relative densities per 100g of all hosts, measurements of the percentage cover of the hosts were obtained for all sites at Nui and Nanumea. Five replicate readings of cover were made using a rope which was marked with 20 random points, recording the algal species found under each point.

MEASUREMENTS OF *CTENOCHAETUS STRIGOSUS* TOXICITY

Ten to fifteen *Ctenochaetus strigosus* were speared at the shallow subtidal areas at the site and time of sampling on all islands, except Nui. Fifty grams of muscle was dissected from each fish and stored in 100% ethanol. Ciguatoxins in these fish samples were confirmed by mouse bioassay (undertaken by E. Shang, Sept,1990). All tissue samples were analysed in the laboratory by the 'Stick-EIA' immunoassay method (Hokama et al.,1983,1987).

ANALYSES

Cell abundance and fish toxicity data were analysed using analyses of variance (ANOVA) with planned comparisons. The main factors examined were: Locations (which were subdivided to examine differences among channels, among controls, between channels and controls, between the wreck and controls, and distances away from channels); Island; Times and Host algae (all fixed). Assumptions of homogeneity of variances were tested using Cochran's test (Winer,1971) and data were transformed $\sqrt{(x+1)}$ as required.

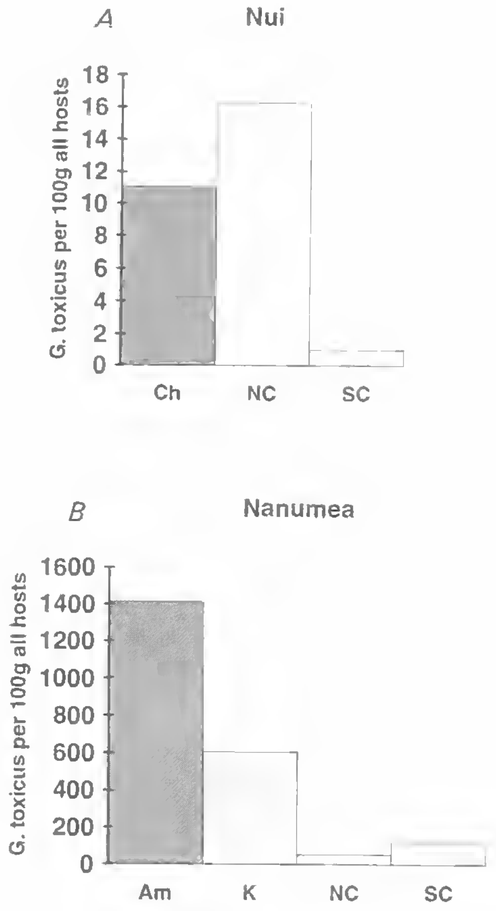

Fig.4. Composite abundances of *G. toxicus* at (A) Nui and (B) Nanumea. Values are calculated as cells per 100g of all hosts (i.e. the relative abundance of each macroalga is taken into account). Ch=Nui Channel, NC=North Control, SC=South Control, Am=American Channel; K=Kennedy Channel.

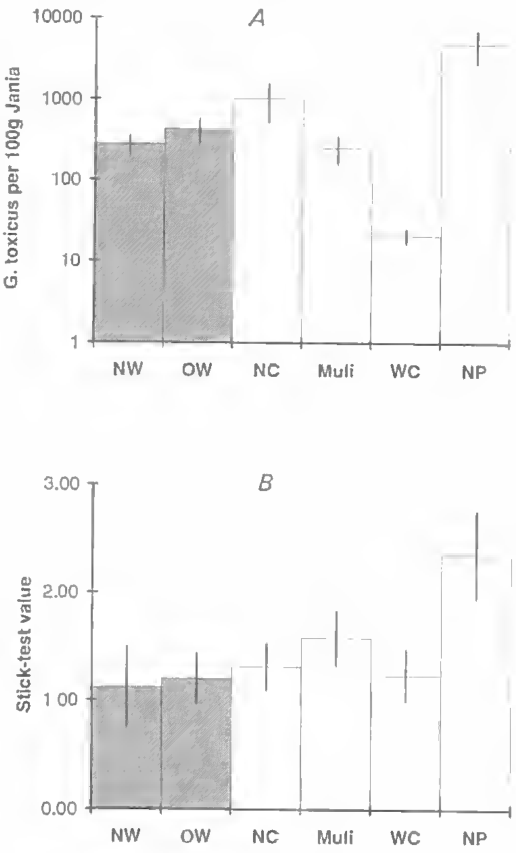

FIG.5. Abundance of *G. toxicus* and fish toxicity at Niutao (Time 2): A comparison of existing wrecks with controls (Note that 'Muli' is regarded as a control for this comparison: at Time 2 channel construction had not yet begun). (A) *G. toxicus* density; (B) Stick test values for fish toxicity. NW=New Wreck, OW=Old Wreck, NC=North Control, Muli=Future site of Muli Channel, WC=West Control, NP=North Point.

RESULTS

PRE-EXISTING BOAT CHANNELS AND WRECKS

Abundance of G. toxicus. There was no difference between the Nui channel and controls in the abundance of *G. toxicus* on *Caulerpa*, *Halimeda* and *Hypnea* (Fig.3A). The numbers of cells recorded was low, with a maximum of 500 cells per 100g of algae on one host at one site only. However, at Nanumea, both channels examined had greater densities of cells than the control areas on *Boodlea* and *Jania* (Fig.3B). For the American passage, densities of cells reached 7000 per 100g of algae. The only alga that could be found at the wreck site was a blue green alga not represented at the channels or controls. Only a few *G. toxicus* were associated with this species at the wreck site.

When cell densities per individual host were adjusted to account for the different percent cover of the algal species, and host algae pooled to provide relative densities of cells per 100g of 'all hosts', a similar pattern was evident. The channel on Nui was intermediate between the two con-

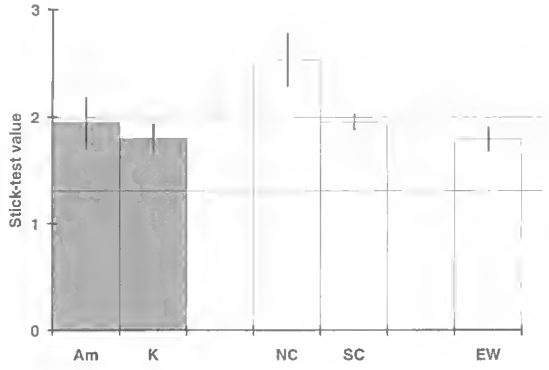

FIG.6. Fish toxicity readings from Nanumea (September 1989=Time 3). Values are means of 10 fish ± standard errors.

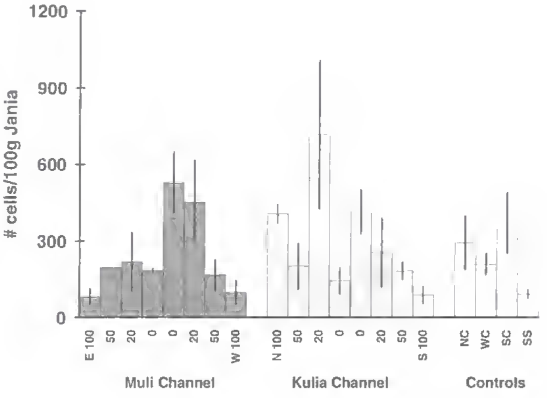

FIG.7. Mean abundance of *G. toxicus* at Channels and Controls at Niutao (Time 3 = October 1989). Values are mean cells counts ± standard errors. At Muli and Kulia, samples were taken at four distances on either side of the channels. At Muli, these were to the East and West of the channel and at Kulia to the North and South. Hence E100 =100m to the East of the Muli Channel etc.

trols (Fig.4A). On Nanumea, densities were considerably higher at channel sites compared to controls (Fig.4B). On average, densities were higher on Nanumea than Nui.

No significant difference could be detected between the 2 wreck sites on Niutao, and 4 controls prior to construction of boat channels (Fig.5A). Cell numbers reached 5000 per 100g of *Jania* at North Point (NP), a concentration similar to the American Passage on Nanumea.

Fish toxicity. There was no effect of boat channels or the wreck on toxicity of *C. strigosus* at Nanumea (Fig.6). Marginally higher toxicity was recorded at the north control site (NC).

Fish collected at wreck sites were no more toxic than control areas on Niutao (Fig.5B). If anything, the means were lower at these sites. The most striking feature of this survey was that the highest level of fish toxicity was recorded at North Point (NP), which corresponded to the highest concentrations of *G. toxicus* in the host alga *Jania*. This was the only site that we consistently found fish giving a positive result to the Hokama immunoassay.

EFFECTS OF NEW BOAT CHANNELS ON NIUTAO

No consistent effect of new boat channels on the abundance of *G. toxicus* was detected (Fig.7, Table 1). While some of the highest densities were recorded at the 0m and 20m sites on the down current side of the channels, most of the channel sites fell within the range recorded at the controls. Patterns of change away from channels indicate that there are major changes in abundance over a 200m distance, but no obvious gradients which could be attributed to channels. Patterns of abundance were so localised that *G. toxicus* abundance on opposite sides of the channels (the 0m distances, only 10m apart) differed at both channels sites.

No significant difference be-

TABLE 1. Analyses of variance of *G. toxicus* cell abundances at Niutao: planned comparisons of channels, controls and distance from channels during Time 3. NW=new wreck; D=distance; Ch=channels; C=controls; NS=non-significant F test; *=p<0.05. Data transformed sqrt(x+1).

FACTOR	DF	SS	MS	DENOM	F	f1,f2	SIG
All cells	62	11270.0	181.77	Res	10.27	62,240	*
All locations	20	7350.68	367.53	S(L)	3.94	20,42	*
Channels	1	191.75	191.75	S(L)	2.05	1.42	NS
Distance	7	4000.21	571.46	S(L)	6.12	7,42	*
Ch x D	7	1990.42	284.35	S(L)	3.05	7,42	*
Controls	3	872.97	290.99	S(L)	3.12	3,42	*
NW vs C	1	163.43	163.43	S(L)	1.75	1,42	NS
Ch vs NW vs C	2	131.94	65.97	S(L)	0.71	2,42	NS
Sample (L)	42	3921.79	93.38	Res	5.28	42,240	*
Residual	240	4246.17	17.69				
Total	302	15516.10					

tween channel and control sites could be found in the toxicity of *C. strigosus* (Fig.8, Table 2). During channel construction (May 1989), 3 months after (September 1989) and 15 months after channel construction, toxicity levels at channels were well within the range exhibited at the controls. In terms of Hokama's thresholds, most fish were within the negative to borderline categories in terms of edibility.

TEMPORAL PATTERNS IN THE OUTBREAK ON NIUTAO

Major changes in the abundance of *G. toxicus* were recorded over the period of this study (Fig.9A, Table 3), being highest at December 1988, and dropping to near 0 at the September 1990. In contrast, fish toxicity levels were lowest at the first sampling date and rose to a peak in September 1989 (Fig.9B, Table 4). This coincided with a severe outbreak of ciguatera on this island (Kaly *et al.* 1991). Toxicity began to decline again in 1991. Although we were not able to continue sampling beyond this date, we have received reports that the outbreak of ciguatera had started to abate by the end of 1992 and fish were being consumed by late 1993. Although data are minimal, there is a suggestion of a lag phase of approximately one year in peak *G. toxicus* numbers and peak toxicity, at least in an indicator herbivorous fish.

DISCUSSION

This study provided no clear support for the view that physical disturbances, such as shipwrecks and boat passages, promote outbreaks of ciguatera fish poisoning. The boat channel on Nui, and the shipwrecks on Nanumea and Niutao all exhibited *Gambierdiscus toxicus* densities and *Ctenochaetus strigosus* toxicity in the range observed at sites several km from these disturbances. However, the results for Nanumea boat channels and trends at Niutao suggest that disturbance may play a role at some times. *G. toxicus* numbers were elevated in the vicinity of boat channels, particularly the American passage, despite the fact that fish toxicity was not. It is noteworthy that the channel area, although not toxic at the time of this study, has been toxic at irregular intervals over the last 20–30 years and at those times was avoided by local fisherman. The channel bisects the reef crest into what was once a ponding lagoon and is a region of extreme currents, and continual disturbance due to wave action. An area considered extremely toxic at the time of this study (on the eastern side of the island) was well-removed from the boat channels.

The before and after study of two new channels

TABLE 2. Analyses of variance of fish toxicity: comparison of channels and controls during T3 at Niutao and Nanumea (Cochran's Q=0.1645, v=14, k=11, NS); NS=non-significant F test; *=p<0.05.

FACTOR	DF	SS	MS	DENOM	F	f1,f2	SIG
Island	1	3.06	3.06	L(IxCh)	4.24	1,4	NS
Ch vs C	1	0.78	0.78	L(IxCh)	1.08	1,4	NS
I x [Ch vs C]	1	1.81	1.81	L(IxCh)	2.50	1,4	NS
Location (I x Ch vs C)	4	2.89	0.72	Res	1.41	4,77	NS
Residual	77	39.44	0.51				
Total	84	47.98					

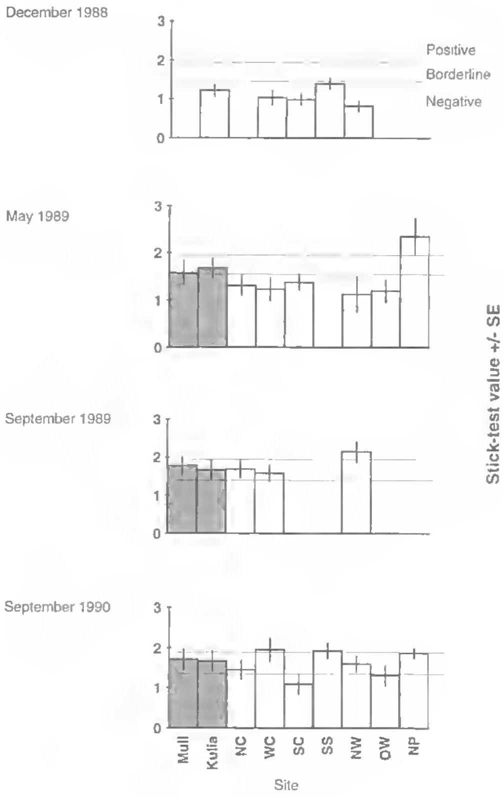

FIG.8. Toxin levels in *Ctenochaetus strigosus* collected at Niutao at 4 times between December 1988 and September 1990. Values are means of stick-test results obtained from 10 fish tested at each site and time ± standard errors. Muli and Kulia are channels, NW and OW are wrecks, remaining sites are controls. Missing bars are unsampled sites, not zero values.

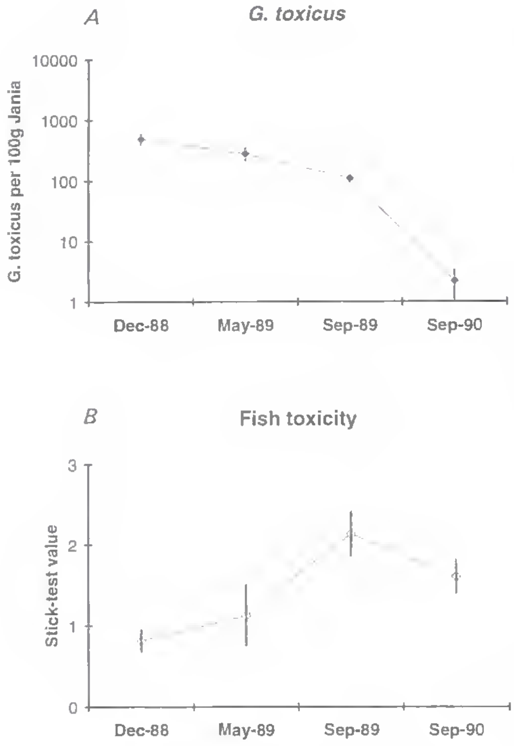

FIG.9. (A) abundance of cells and (B) levels of fish toxicity through time at 'New Wreck', Niutao.

on Niutao did not indicate a successional change in macro-algal hosts and an early successional outbreak in *G. toxicus*, although numbers appeared to be slightly elevated on the down-current side of each channel. However, cell numbers in the vicinity of channels were not unusually high relative to control areas at any stage during the study. Channel construction on this island was preceded by a bloom of *G. toxicus* which must have been caused by some other phenomenon (Kaly et al. 1991). This may have made any effect of channels difficult to detect.

Physical disturbance to reefs may or may not lead to ciguatera. 'Disturbance' encompasses a host of phenomena which may affect the habitat and population dynamics of *G. toxicus* in different ways. Whether or not ciguatera is induced by disturbance may depend on the intensity, timing, frequency and scale of the disturbance, all factors known to affect the trajectory of successional patterns on hard substrata (Connell & Keough, 1985). The time-scale for recovery may depend on the regime of disturbance and chance factors in the recolonisation of damaged areas. Disturbance itself may interact with other phenomena to explain the observed patterns of outbreaks. Factors such as wave exposure, fresh water run-off and coral destruction are likely to be closely linked, making such interactions likely and single-cause scenarios extremely unlikely.

Different intensities of disturbance may have opposite effects on ciguatera. If coral habitat is damaged and the growth of host algal species promoted, then densities of *G. toxicus* could be elevated. Seasonally high wave exposure and wind at North Point (NP) on Niutao appeared to correlate with high numbers of *G. toxicus*, which also coincided with a higher average fish toxicity. In other studies, moderate wave exposure has acted to reduce cell densities, presumably by dislodging cells without disrupting the habitat (Anderson & Lobel 1987).

A promising area for research lies in the potential relationship between *G. toxicus* numbers and fish toxicity. While there was some evidence that the distribution of *G. toxicus* around Niutao correlated with fish toxicity, toxic fish were caught all round the island during the peak of the outbreak (Kaly et al. 1991). Temporal monitoring during this outbreak was limited but suggested a lag phase of approximately one year between peak *G. toxicus* numbers and the highest toxicity levels. More comprehensive sampling is needed to confirm this pattern. However, if such a pattern proves to be of general significance there are obvious implications with regard to the forecasting and managing of ciguatera outbreaks.

TABLE 3. Analyses of variance of *G. toxicus* cell abundances at Niutao: comparison of 2 hosts (*Jania* sp. and unidentified green) through time at New Wreck (NW). NS=Non-significant F test; *=p<0.05. Data transformed sqrt(x+1).

FACTOR	DF	SS	MS	DENOM	F	f1,f2	SIG
Times	3	4453.72	1484.57	S(Ti x H)	77.83	3,12	*
Host	1	968.58	968.58	S(Ti x H)	50.78	1,12	*
Ti x H	2	500.31	250.15	S(Ti x H)	13.11	2,12	*
Sample (Ti x H)	12	228.91	19.08	Res	3.46	12,69	*
Residual	69	380.35	5.51				
Total	86	3718.46					

TABLE 4. Analysis of variance of fish toxicity: comparison through time of Kulia, New Wreck and West control at Niutao (Cochran's Q=0.1399, v=9, k=12, NS). NS=non-significant F test; *=p<0.05.

FACTOR	DF	SS	MS	DENOM	F	f1,f2	SIG
Time	3	14.66	4.89	Res	8.99	3,103	*
Location	2	0.94	0.47	Res	0.86	2,103	NS
T x L	6	4.70	0.78	Res	1.44	6,103	NS
Residual	103	55.95	0.54				
Total	114	76.25					

Tukey's Test: T3 (Sep 89) T4 (Sep 90) T2 (May 89) T1 (Dec 88)

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